The relationship between elevated fibrinogen and markers of infection: a comparison of seasonal cycles

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Summary

To test the hypothesis that higher levels of fibrinogen in winter are related to infections via the acute phase response, we assessed seasonal variation in fibrinogen and C-reactive protein, together with three other responses to infection: white cell count, human herpesvirus-6 IgG antibody and interleukin-6. Monthly blood samples from 24 subjects aged 75+ years were assessed for fibrinogen, C-reactive protein, white cell count, and human herpesvirus-6 IgG antibody. Interleukin-6 was measured in seven. Seasonal variation of these measures was determined by the population-mean cosinor procedure. Fibrinogen had a significant seasonal variation with a winter peak (mid-February) 1.26 g/l above the corresponding summer trough. C-reactive protein had a late-February peak, 3.71 mg/l above the summer trough. No seasonal rhythm was found in any other response to infection investigated. This study provides no evidence that winter infections are responsible for the seasonal variation in fibrinogen or C-reactive protein. The explanation for the seasonal changes in these proteins remains unknown.

Introduction

A possible explanation for the winter increase in cardiovascular morbidity and mortality,1 is a seasonal effect on cardiovascular risk factors. Three recent longitudinal studies of older people living in the community, have measured a number of possible risk factors over at least one annual cycle.2–4 These studies have identified a marked seasonal variation in fibrinogen with highest levels in winter. Fibrinogen is a major risk factor for cardiovascular disease,5–8 and is also an acute-phase protein9 which may increase in response to infections and other stressful events. Increasingly, reports have linked infections to atherosclerosis and thrombosis.10–12

The role of fibrinogen in the acute-phase reaction offers a biologically plausible hypothesis for the involvement of infection and inflammation in cardiovascular disease. Two of the longitudinal studies found no association between the winter elevation of fibrinogen and some concomitant markers for infection,2,4 while the third3 suggested that upper respiratory infections might be related to higher fibrinogen levels in winter. We investigated the potential role of infection further, by determining in the same individuals, annual rhythms of fibrinogen, C-reactive protein (CRP), the major acute-phase protein, and several other responses to infection, namely: white cell count (WCC); human

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herpesvirus-6 IgG (HHV-6) and interleukin-6 (IL-6), a key cytokine in the inflammatory response.\textsuperscript{13}

**Methods**

**Subjects and measurements**

Measures for fibrinogen, CRP and WCC were available from our original study of 68 healthy individuals aged 75 + years.\textsuperscript{2} These people were identified from the register of a local general practice, and were included in the study following an initial health assessment. This excluded individuals with existing medical conditions or who were taking medications which may have affected the measurements of interest. All subjects gave written informed consent. Monthly blood samples were obtained from an antecubital vein and transported immediately to the laboratory. The research nurse noted if the subject had been unwell prior to blood-taking, and visits were arranged for the same weekday and time of day on each visit to minimize circadian and weekday variation. Plasma fibrinogen (g/l) was determined by the Clauss method;\textsuperscript{14} WCC ($10^9$/l), and CRP (mg/l) were measured by routine automated laboratory tests. Since these measures were determined in fresh blood, strict laboratory standardization procedures were maintained throughout the study, in order that any patterns observed in the data were not artefacts of variation in analytical procedures, reagents or instrumentation. All 68 subjects from this original study had seven or more monthly readings for fibrinogen and 85% had $\geq$ 10 readings. Only 24 subjects however had 12 consecutive monthly values for fibrinogen, and all of these people were selected for the current investigation. Given the statistical methodology used, the selection of individuals with data spanning one complete annual cycle was appropriate. Table 1 compares the characteristics of this selected group of 24 with those of the 44 not included. The present study therefore used existing values for fibrinogen, WCC and CRP which had been determined in fresh blood at the time of the original study and measured IL-6 and HHV-6 reactivation in stored plasma from the same samples. Both IL-6 and HHV-6 antibodies are considered stable in plasma frozen at $-70^\circ$C. HHV-6 reactivation was determined by assay of HHV-6 IgG by indirect immunofluorescence.\textsuperscript{15} The level of fluorescence was recorded in a semi-quantitative manner from negative to strongly positive through three ascending intermediate levels. IL-6 (pg/ml) was measured by an ELISA system using 100 µl aliquots which had no freeze-thaw cycle\textsuperscript{16} in seven of the 24 subjects who had shown the largest seasonal variation for fibrinogen and who had nine or more monthly values.

**Statistical analysis**

Mean monthly values were determined for each measure, to allow visualization of the crude data. Seasonal rhythms were detected and quantified by the population-mean cosinor procedure.\textsuperscript{17,18} This involves fitting single cosinor models for each variable and for each individual. This single cosinor mathematical model assumes that the outcome variable follows a sinusoidal curve with a period of one year. The parameters measured by the model are as follows: (i) Mesor ($M$), the value about which the oscillation occurs; a rhythm-adjusted mean; (ii) Amplitude ($A$), the difference between $M$ and the highest or lowest fitted value, the seasonal variation being twice the amplitude; (iii) Acrophase ($\phi$), the interval between zero time and peak value in terms of calendar months, measured in negative degrees clockwise from the reference point of 1 January. The seasonal parameters resulting from these individual models are then addressed mathematically using the population-mean cosinor methods to give population parameters for the variables. In this analysis, statistical tests are based solely on the variability among individual parameter estimates. The null hypothesis that no seasonal variation exists, corresponds to the amplitude being equal to zero in the model. The significance of the amplitude of the population rhythm is determined

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Study group characteristics comparing the 24 subjects included in the present study with the 44 subjects remaining</th>
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<tbody>
<tr>
<td></td>
<td>Subjects remaining ($n = 44$)</td>
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<tr>
<td>Age (years)</td>
<td>81.93 (5.51)</td>
</tr>
<tr>
<td>Mean fibrinogen (g/l)</td>
<td>3.03 (0.73)</td>
</tr>
<tr>
<td>Mean CRP (mg/l)</td>
<td>6.72 (6.39)</td>
</tr>
<tr>
<td>Mean WCC ($10^9$/l)</td>
<td>7.02 (2.83)</td>
</tr>
</tbody>
</table>

Mean and (SD) determined using all monthly readings; $t$-test with 66 d.f.
by the F statistic. The methods of cosinor rhythmometry are statistically very powerful. When measurements are assigned more or less evenly along a full cycle of periodicity (in this case 1 year), 80% power can be reached to resolve the rhythmic structure of the data based on a relatively small sample (k ≥ 3).

Results

Visualization of the crude data using mean monthly values for fibrinogen and CRP showed a seasonal fluctuation with higher values in winter, while other markers for infection showed no seasonal pattern (Figure 1).

Quantification of the seasonal changes by the population-mean cosinor procedure determined that the population comprising 24 individuals exhibited a highly significant seasonal amplitude of 0.63 g/l in fibrinogen, with the peak occurring in mid-February (Table 2). This represents a seasonal variation (SV) of 1.26 g/l. CRP in this same population also varied seasonally with a late-February peak and an amplitude of 1.86 mg/l (SV 3.72 mg/l). No significant seasonal rhythm was present in the population for the other markers of inflammation investigated (Table 2). Individual rhythms of the markers of infection IL-6, WCC and HHV-6 exhibited extreme phase differences, and peaks occurred throughout the annual cycle with no clustering in any given month or annual quarter. (Figure 2b). Conversely, individual fibrinogen peaks all occurred in the first quarter of the cycle.

![Figure 1](image)

**Figure 1.** Mean monthly values for seasonal measurements (a) and non-seasonal measurements (b).

| Table 2 | Annual rhythm parameters determined by the population-mean cosinor procedure for fibrinogen, C-reactive protein, white cell count, human herpesvirus-6 and interleukin-6 |
|---|---|---|---|---|---|---|
| **n** | **Mesor (M)** | **Amplitude (A)** | **F-statistic** | **p** | **Seasonal variation** | **Acrophase (ϕ)** |
| Fibrinogen (g/l) | 24 | 3.07 (2.88–3.26) | 0.63 (2.44–3.70) | 76.15 | <0.0001 | 1.26 | –43.61° | Mid-February |
| C-reactive protein (mg/l) | 24 | 6.40 (4.75–8.06) | 1.86 (4.55–8.30) | 7.94 | <0.005 | 3.72 | –50.14° | Late-February |
| White cell count (10⁶/l) | 24 | 6.32 (5.79–6.85) | 0.21 (6.11–6.53) | 1.945 | NS | – | – |
| Human herpesvirus-6 (au) | 24 | 3.03 (2.76–3.30) | 0.07 (2.96–3.10) | 0.655 | NS | – | – |
| Interleukin-6 (pg/ml) | 7 | 7.91 (5.11–10.71) | 1.24 (6.67–9.15) | 0.56 | NS | – | – |

* Rhythm trough to rhythm peak.
and individual C-reactive protein peaks clustered around March (Figure 2a).

**Discussion**

Fibrinogen levels in the population studied in this investigation show a clear seasonal rhythm, with highest levels recorded in mid-February. This confirms our original report\(^1\) in which a less sophisticated statistical analysis was used, and those of Woodhouse \etal. (1994)\(^3\) and van der Bom \etal. (1997).\(^4\) The seasonal difference detected in fibrinogen in this study (1.26 g/l), and the others (Woodhouse 0.13 g/l in 100 subjects aged 65–74; van der Bom 0.34 g/l in 2325 subjects aged

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**Figure 2.** Vectorial representation of individual seasonal rhythm parameter estimates, amplitude (A) and acrophase (ϕ) resulting from the single cosinor model. Each rhythm is resolved into two components, β and γ, where β = A cosϕ and γ = −A sinϕ in the direction at right angles. The resultant vector gives both A as the distance from the origin (0,0) and ϕ as the angle from 0° (January 1), with 360° representing one annual cycle. Those measurements determined to have significantly seasonal variation in the population are shown in a, while those with no significant rhythm are shown in b.
55+ years, rising to 0.43 g/l in subjects aged 75+ years), represents a clinically important elevation with the potential to increase death from ischaemic heart disease by 15–144%.\textsuperscript{20} The variation in size of the seasonal variation reported by each study is not surprising given that each was carried out in a different annual cycle, in a different population and using different analyses. The important fact remains, however, that fibrinogen does vary seasonally with sufficiently large amplitude to increase cardiovascular risk in winter months.

C-reactive protein is the major acute-phase protein in humans, with concentrations rising several-hundred-fold in acute illness.\textsuperscript{21} Low levels of chronic inflammation may produce a rise similar to that seen during acute illness.\textsuperscript{22} In our original study, we reported that C-reactive protein showed no significant seasonal change.\textsuperscript{6} However, the sensitivity of the current statistical analysis revealed a significant seasonal rhythm in the 24 subjects selected for the current study. The magnitude of this winter/summer change in CRP (3.72 mg/l) is however relatively small in comparison to the several-hundred-fold rise which may occur in response to acute infection or chronic inflammation. It is interesting that in the present study, the peak concentration of C-reactive protein is attained around 1 week after the peak for fibrinogen (using an approximation of 1° per day); a reversal of what would normally be expected in the sequence of an acute-phase reaction.

Raised white cell count is a general indicator of the presence of infection and levels of the pro-inflammatory cytokine IL-6 increase rapidly in body fluids after infection. The importance of IL-6 as an inducer of the acute-phase response has been confirmed by the observation that it induces acute-phase proteins in vivo.\textsuperscript{23} Reactivation of the common latent virus HHV-6 occurs in the presence of other infections\textsuperscript{24} and in addition, HHV-6 is similar both morphologically and in its genomic homology with cytomegalovirus, a seasonal virus exhibiting a December peak which has been linked to cardiovascular disease.\textsuperscript{25} Cytomegalovirus however is present in only 40% of the population, while HHV-6 exhibits a higher infection level, with 66% of adults age 40 years and over showing seropositivity for the virus. HHV-6 was therefore chosen as a surrogate marker which would reflect infective episodes in the population cohort. The inability or failure to show an association, would indicate a lack of association or sub-optimal surrogacy. HHV-6 antibody levels decline with age,\textsuperscript{24} so a finding of high IgG levels in an elderly population would suggest reactivation resulting from an infection.

WCC, HHV-6 and IL-6 in the same individuals who had shown a highly significant annual variation in fibrinogen, showed no seasonal change. These results, and those of other studies,\textsuperscript{4,26} contrast to some extent with those of Woodhouse et al. (1994), who reported significant seasonal variation in infection levels, associated with fibrinogen changes, as measured by self-reported cough and coryza, WCC and measures of the acute-phase reactants CRP and α1-antichymotrypsin.\textsuperscript{3} These measurements peaked in March and April, in contrast to the fibrinogen peak in February.

If elevation of WCC and IL-6, or reactivation of HHV-6, did not vary seasonally because our population was not exposed to more infection during the winter months of the study, then an explanation for the highly significant and large seasonal variation in fibrinogen and CRP is still required. We of course cannot rule out the possibility of infections from organisms which we have not studied. Other seasonal factors should be investigated. So far no clue to the identity of the stimulus for the seasonal variation of fibrinogen has emerged.

**Conclusion**

Seasonal variation in fibrinogen has been shown in several studies, but the mechanism by which this occurs remains unknown. The results reported here do not support the hypothesis that the seasonal rhythm in fibrinogen results from an acute-phase reaction initiated by infections. An explanation for the seasonal changes in fibrinogen remains to be found.

**References**